oocyte *in vitro* is provided at e.g., p. 65, second paragraph. New claim 128 is similar to claim 123 except that it does not include the steps of maturing the zygote to an embryo and introducing the embryo into a recipient female bovine. The zygotes resulting from the method of claim 128 can be stored frozen and matured to bovines at a later time, if desired.

Claims 82, 83, 97-118, 123, 125, 126 and 128 are pending. The Examiner's specific comments are now addressed in turn.

Claims 73 and 120: The rejections of these claims at p. 2 of the office action have been mooted by cancellation of the claims without prejudice.

Rejection of all claims under §112, first paragraph

The Examiner says that it is well known in the art that the level and specificity of expression and the resulting phenotype are dependent on the transgene used. The Examiner states that the individual gene of interest, promoter, enhancer, coding or noncoding sequences, site of integration, and methylation inactivation are all important factors in controlling expression. The Examiner cites Palmiter and Kappel as discussing the effects of introns and methylation on expression. The Examiner cites Sharmay as discussing a situation in which transgenic expression of an acid whey gene impaired mammary gland development in some pigs. The Examiner states that the specification provides insufficient guidance to prepare transgenic bovines species containing all mammary gland promoters enhancers and secretory sequences, and therefore that the disclosure is enabling only for specifically disclosed transgenes.

It is noted that all of the Examiner's reservations as to enablement concern whether expression of a transgene will be obtained. **However, the broadest claims do not specify that the transgene must be expressed.** Rather, the claims specify only that the transgene is integrated into a recipient genome. Bovines produced by the claimed methods that contain an integrated nonexpressed transgene are, of course, useful for the same purposes as nontransgenic bovines are useful (e.g., producing dairy products). "Nothing in the patent statute requires than an invention be superior to the prior art to be patentable." *Ryco Inc. v. Ag. Bag Corp*, 8 USPQ2d 1323, 1328

(Fed. Cir. 1988). In addition, the existence of the integrated transgene provides a marker by which bovines can be readily identified. Because claims 123, 126 and 128 do not require expression, and all of the Examiner reservations concern expression, the rejection is inapplicable to these claims.

Even for the claims that do specify that the transgene is expressed, it is respectfully submitted that the Examiner is applying an unduly high standard of enablement. Enablement does not require universal applicability, but rather, at most, general applicability. "Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. It is not the function of the claims to specifically exclude possible inoperative substances." *Atlas Powder Co. v. Du Pont De Nemours & Co.*, 244 USPQ 409, 414 (Fed. Cir. 1984). Further, the courts have recognized that in some fields even substantial screening of products having desired characteristics from a large primary pool with its inevitable proportion of negative results is expected and routine to one of ordinary skill. *In re Wands,* 8 USPQ2d 1400 (Fed. Cir. 1988). In *Wands*, the claims specified IgM monoclonal antibodies having a binding affinity of $\leq$ 1 nM. The court noted that identification of antibodies falling within the claims would require (1) producing monoclonal antibodies against a specific antigen, (2) qualitative screening for binding affinity to the antigen, and (3) quantitative measurement of dissociation constant for each antibody. Nevertheless, the court found that such screening did not constitute undue experimentation, and hence that the claims were patentable.

Here, the application provides a general method for generating transgenic bovines that is quite independent of the transgene being introduced. See declaration of Dr. Janne at paragraph 13 ("It is clear that [the invention] has general applicability to generate any type of transgenic bovine"[1]). If obtaining expression is one's ultimate goal, the method can be performed on a sufficiently large-scale to generate a primary

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[1]The declaration was originally submitted in the corresponding European application, No. 91 901026.4. Note that USSN 07/444,745, filed Dec. 1, 1989, referenced at the top of the Janne declaration, is an ancestor application from which the present application derives priority. A copy of Professor Janne's curriculum vitae will be filed under separate cover.

collection of transgenic bovines from which it is probable that at least one will provide satisfactory expression. Expressing bovines can easily be distinguished from nonexpressors (e.g., by Northern blotting of biopsied tissue). Further, although the site of integration of a transgene may be unpredictable, the influence of the site on expression can be minimized by following the teaching of the specification that large flanking regions are preferred to insulate a transgene from localized effects of surrounding chromatin (see, e.g., specification at pp. 18-20).

That the description of the present specification is sufficiently detailed to guide practitioners to successful application of the claimed methods in several instances is abundantly clear from the declaration of Dr. Janne and publications describing transgenic bovines generated by these methods after the priority date of the present application (December 1, 1989) and after its publication by the present inventors in Krimpenfort et al., *Bio/Technology* 9, 844-847 (1991). Later publications reporting additional examples of transgenic bovines produced by *in vitro* methods include Bowen, *Theriogenology* 39, 194 (1993) and *Biology of Reproduction*, 50, 664-668 (1994); Hyttinen et al., *Bio/Technology* 12, 606-608 (1994); and Hill et al., *Theriogenology* 37(1), 222 (1992).[2] The Examiner's attention is also drawn to Professor Janne's comment that "...the efficiency of the *in vitro* methods will ensure success by routine repetitions of the same procedures" (declaration at paragraph 12).

The Examiner has not commented on the detailed guidance provided by the specification, but instead focuses on references discussing uncertainties as to the levels of transgenic expression in some instances. These uncertainties are insufficient to establish serious doubt that at least one transgenic animal showing at least some expression can be obtained with a reasonable amount of screening by using the claimed methods (assuming that obtaining expression is one's goal). The present claims do not specify a particular level of expression.

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[2]Copies of these references will be provided under separate cover.

For example, although three of six of Sharmay's transgenic pigs bearing an acid whey transgene showed impaired mammary gland development, the other three pigs showed normal development and transgene expression and could easily be distinguished from the abnormal pigs. Moreover, strictly speaking, the impaired mammary gland development was a consequence of expression rather than lack of expression. In any event, 50% of the pigs in Sharmay's experiment did show normal mammary gland development and protein expression in the milk. With 50% of transgenic animals generated having the desired characteristics, screening for these animals is not an intolerable burden.

Kappel states that methylation patterns *may* alter the pattern of chromosome expression in certain regions of the chromosome. However, Kappel does not state that methylation patterns completely eliminate expression. Further, Kappel does not provide any indication that the regions affected by methylation constitute a substantial proportion of the chromosome. Kappel also does not address whether host chromosomal patterns are reconstructed in a transgene notwithstanding the inclusion of substantial length of flanking DNA in the transgene unrelated to the endogenous sequences. In these circumstances, Kappel provides insufficient evidence to generate a reasonable concern that the effects of methylation will be so frequent or severe in impairing gene expression as to make the screening required to generate an expressing transgenic animal an undue burden.

Palmiter's observation that higher levels of expression are obtained from genomic constructs or the inclusion of intronic sequences echoes the teaching of the present application (see, e.g., p. 23, 2nd paragraph). Based on the present application, a practitioner having an objective of obtaining high expression would know to use a genomic sequence and/or include an foreign intronic sequence from a highly expressed gene between the promoter and coding sequence (such as the Ig hybrid intron exemplified in the specification). Palmiter also discusses experiments designed to identify which introns are most effective in increasing expression and which positions relative to the coding sequence are most effective for such introns. Although this kind of information might be a subject of academic interest to some, it is not an obligatory preliminary to practicing the

claimed methods, when sources of DNA likely to give good results (i.e., whole genomic fragments and/or fragments including an Ig hybrid intron between coding sequence and promoter) are readily apparent and available. Furthermore, although Palmiter tries a large number of sources, permutations and positions of intronic sequence, the vast majority of constructs were expressed to some degree. These results are typical of the general experience in the art in generating transgenic animals; that is, while the levels of expression may vary between different animals, the vast majority of transgenic animals show detectable expression of the transgene.

For these reasons, it is respectfully submitted that the claimed methods generate transgene-expressing bovines (if this is one's goal) in sufficient frequency for them to be identified by routine repetition of the method steps and a tolerable amount of screening. The law requires no more.

Art-based Rejections

1. Rejection of claims 120 and 121 as anticipated or obvious over Meade or Hopp or Gordon

This rejection has been mooted by cancellation of claims 120 and 121.

2. Rejection of claims 122-127 as obvious over Meade, Hopp or Gordon taken with First

The Examiner says that Meade, Hopp and Gordon each disclose a method for producing a transgenic mammal capable of producing a human serum protein in its milk, but do not specifically teach the preparation of a transgenic cow or the *in vitro* fertilization and culturing of bovine ova into implantable embryos. The Examiner states that First discloses a method of *in vitro* fertilizing and culturing bovine ova into implantable embryos. The Examiner takes the view that it would have been obvious to modify the teachings of any of Meade, Hopp or Gordon by introducing a human serum protein gene into bovine embryos which have been fertilized *in vitro* to obtain transgenic cows capable of producing a human serum protein in their milk with a reasonable expectation of success. This rejection is respectfully traversed.

Attached is an declaration by Professor Neal First providing an expert opinion that the claimed methods represent a substantial advance in the art, and that their successful practice could not have been reasonably expected from, e.g., US 5,213,979 (the '979 patent).[3] This opinion merits particular respect not only because Professor First is a renowned scientist in the field of embryology, but also because he is a co-inventor of the '979 patent and highly familiar with the work described therein. Although the declaration speaks for itself, the reasoning underlying Professor First's opinions is briefly outlined below.

In Professor First's opinion, the claimed methods represent a substantial advance in the art relative to the '979 patent, in part, because the claimed methods made possible a wider range of genetic manipulations than that performed in the '979 patent.

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[3]An unsigned declaration accompanies this response. A signed version will be provided under separate cover.

Professor First explains that the goal of the '979 patent was the manipulation of blastocyst-stage embryos (as described in column 1, lines 55-60). For example, this procedure would expedite the generation of herds of genetically superior animals (particularly dairy cattle) by allowing cloning of a blastocyst believed to have desirable naturally occurring characteristics. Professor First contrast this specific goal with that attained by the claimed methods, which allow introduction of any transgene into a zygote leading to phenotypes not found in nature. Professor First emphasizes that the '979 patent did not exemplify or otherwise describe any manipulations involving introduction of transgenes into embryonic cells (First declaration at paragraph 5).

Professor First provides detailed reasoning to explain his view that the success of the claimed methods was not reasonably expected from the '979 patent. Professor First begins by discussing the failures and frustrations of previous attempts to generate transgenic cattle. Professor First also notes that in these unsuccessful attempts, as well as in methods of transgenesis for mice (e.g., as discussed in Meade, Hopp and Gordon), oocyte maturation and fertilization were performed *in vivo*. Professor First then lists a number of problems and uncertainties in combining the *in vitro* procedure of the '979 patent with traditional mouse transgenesis techniques or the unsuccessful bovine methods of Biery, Loskutoff and Bondioli. For example, Professor First discusses the impact of differences in physiology and cell phase between *in vivo* and *in vitro* mature oocytes on a transgenesis protocol. Professor First also notes uncertainty whether the 8-cell stage block on bovine embryo development could be removed in the context of a transgenesis protocol.

In Professor First's expert opinion, the difficulties and uncertainties would have been expected to require considerable experimentation to perfect or overcome. The requirement of considerable experimentation would have been particularly onerous in the present circumstances in view of Professor First's further observation that the ultimate end point for determining the efficacy of such experimentation (i.e., the production of a viable transgenic bovine) would not have been apparent until several years later. Thus, Professor First concludes that it was

not practically possible to vary systematically most of the parameters; thus, explaining his view that the success of the claimed methods was not reasonably expected.

Many of the points made by Professor First are reiterated in the attached declaration by Professor Janne. Professor Janne is also a distinguished scientist who has published over 200 articles, and has worked in the field of transgenesis since 1988. In paragraph (5) of his declaration, Professor Janne discusses the failures of previous attempts to generate viable transgenic bovines, such as described in the Biery and Loskutoff references. Professor Janne notes that these references used oocytes fertilized *in vivo*, a step, which Professor Janne describes as a "bottleneck" in the entire procedure (declaration at paragraph 6). Dr. Janne then considers protocols for *in vitro* maturation for bovine oocytes that had been reported at the priority date of the present application, and concludes that "there was no reasonable expectation of success in using immature oocytes as a source for the generation" (declaration at paragraph 7).

Professor Janne provides a detailed discussion of the factual basis for this opinion. Professor Janne notes that the efficiencies of *in vitro* maturation procedures in 1989 were low and variable. Dr. Janne then discusses differences in physiology and cell-cycle phasing between *in vivo* and *in vitro* matured oocytes, reiterating some of the grounds for Professor First's opinion.

That others hold similar views to Professors First and Janne regarding the substantial and dramatic advance of the claimed methods in a difficult and unfruitful field is illustrated by the following comments.

> The commercial development of transgenic bovine technologies, however, has been frustrated because the protocols used successfully with smaller animals--which require large numbers of embryos and several surgical procedures-are prohibitively expensive when applied to cattle. The establishment of an *in vitro* embryo production system, as described by Herman de Boer and his coworkers at Gene Pharming Europe[4]...is therefore a dramatic breakthrough in enlarging the transgenic pharm-yard.

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[4]The assignee of the present application, Pharming B.V. was formerly known as Gene Pharming Europe B.V. Gene Pharming Europe B.V. was formerly a subsidiary of GenPharm International Inc.

Bialy, *Biotechnology* 9 (1991).

> A group in the Netherlands reports in a paper in the September issue of Bio/Technology successful generation of the first transgenic dairy calf, which carries a gene for production in cow milk of human lactoferrin (HLF)...GenPharm came up with a novel integrated *in vitro* process for generating transgenic cattle.

Selzer, *Chemical & Engineering News* 69, 7 (1991).

> But historically, efforts to produce transgenic dairy cows have been thwarted because of cumbersome and costly surgical procedures. Now, however, researchers from Gene Pharming Europe.... have circumvented the need for surgical removal and transfer of embryos by combining gene transfer with an *in vitro* embryo production system.

Gershon, *Nature* 353, 7 (1991).[5]

It is respectfully submitted that these remarks by third parties and the declarations of Professors First and Janne establish the patentability of the pending claims and shift the burden to the PTO to rebut with appropriate *evidence*.

> [W]hen an applicant demonstrates *substantially* improved results...and *states* that the results were unexpected, this should suffice to establish unexpected results *in the absence of* evidence to the contrary.

In re Soni, 34 USPQ2d 1684, 1688 (Fed. Cir. 1995) (emphasis in the original).

> Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Guidelines for Examination of Applications for Compliance with the Utility Requirement at § B.4

Here, Applicants have provided a reasoned opinion by Professor First that the claimed invention represents a substantial advance in the art. Moreover, this is a widespread opinion in the art as illustrated by the quotations from third parties listed above. Further, Professors First and Janne have not only *stated* that the success of the claimed methods was unexpected from the cited art, including the '979 patent, but have provided detailed reasoning in support of these opinions. By contrast, the office action has provided only a conclusionary assertion that a reasonable

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[5]Copies of these references will be provided under separate cover.

expectation of success would have followed from the teachings of the cited references. The office action has not considered any of the facts underlying the expert opinions of Professors First and Janne, much less provided *evidence* to controvert their accuracy. Nor has the office action taken into account the acclamation of the art in describing the claimed methods *inter alia* as a "dramatic breakthrough." In these circumstances, the PTO must defer to the conclusions of the distinguished experts, Professors First and Janne, from which it follows that the claimed methods were not obvious.

3. Rejection of claims 73, 82, 83, 97 and 98-121 over Meade, Hopp, or Gordon taken with any one of Loskutoff, Biery or Bondioli. Claims 73 and 119-121 have been cancelled. The other claims have been made dependent directly or indirectly on claim 123 which was not subject to this rejection. Thus, the rejection is moot.

4. Rejection of claims 122-127 as obvious over Meade, Hopp, Gordon when taken with Loskutoff, Biery or Bondioli in further view of First. This ground of rejection appears to be similar to rejection (2) above except that Biery, Bondioli and Loskutoff are also cited. The teachings of these references were discussed in the declarations by Professors First and Janne and were taken into account in the opinions expressed. Thus, it is submitted that this ground of rejection is fully addressed by the above remarks.

5. Rejection of claims 73, 82, 83 and 97 and claims 98-121 as obvious over any one of Simons, Clark, Gordon, Bremel, when taken in view of any one of Loskutoff, Biery or Bondioli. Claims 73 and 119-121 have been cancelled. The other claims have been made dependent directly or indirectly on claim 123 which was not subject to this rejection. Thus, the rejection is moot.

6. Rejection of claims 122-127 as obvious over any one of Simons, Clark, Gordon or Bremel when taken with any one of Loskutoff, Biery or Bondioli in further view of First. This rejection appears to be similar to rejection (4) above except that Simons, Clark or Bremel are cited as alternative references

to Gordon. The Simons and Clark references discuss experiments to generate transgenic sheep. In these experiments, as in the traditional mouse transgenesis procedures referred to in Gordon, maturation and fertilization of oocytes occurred *in vivo*. Bremel is a general review article and discusses traditional transgenesis methods in mice and sheep, such as those discussed by Gordon, Simons and Clark. In brief, it is submitted that these references are substantially cumulative with Gordon. Thus, above rejection is fully addressed by the remarks under (2) and (4) above.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (415) 326-2400, Ext. 218.

Respectfully submitted,

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Attachments:
Declaration of Professor First
Declaration of Professor Janne

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